The Journal of Trace Elements in Experimental Medicine 13:205–213 (2000)

Mineral and Trace Elements in Meconium: Comparison in Dizygotic Twin Pairs

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It has not been determined whether the concentration of essential elements in the meconium of dizygotic twins is indistinguishable or is related to birth weight or placental size differences. We hypothesized that dizygotic twins of discordant size would have a dissimilar concentration of essential elements in the meconium, whereas concordant twins would not exhibit such a difference. We also hypothesized that the smaller the infant/placental weight ratio, the greater the concentration of essential elements in meconium. This study was aimed at verifying these hypotheses. Twenty-six pairs of dizygotic twins, regardless of sex, were divided into two groups separated by the median value of weight discrepancy (13.1%) into concordant (CC) $\leq$13.1% and discordant (DC) >13.1%. The concentrations of seven essential elements (calcium, magnesium, phosphorus, copper, zinc, iron, and manganese) were measured in the meconium. Total placental weights for all twins were also correlated with infant weights and meconium element concentration. Heavy/light birth weight ratio of the DC, but not of CC twins, corresponded to a meconium concentration ratio >1 for calcium, magnesium, iron, zinc, and manganese, but not for phosphorus and copper. There was a strong correlation between total twin weight and total placental weight for both the CC and DC groups. However, infant/placental weight ratio did not correlate with meconium element concentration. It may be inferred that in dizygotic twins, a higher essential element concentration in meconium may reflect the greater access of the heavier fetus to maternally supplied nutrients, or a different functional capacity of the gastrointestinal tract. J. Trace Elem. Exp. Med. 13:205–213, 2000.

Key words: multiple births; dizygosity; placenta; birth weight

INTRODUCTION

Inorganic elements constitute about 6% of the human body weight. Essential trace elements are a small fraction of this total [1,2]. During gestation, the fetus is dependent upon a continuous supply of organic and inorganic substances. The latter are necessary for bone formation, enzyme synthesis, fluid balance, and virtually every vital process. The placenta is highly vascularized by maternal and fetal vessels and functions as the regulatory organ of maternal-fetal exchange [3]. Mineral elements are

Contract grant sponsor: North Shore University Hospital House Staff Grants Program.

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Received 4 November 1998; Accepted 22 April 1999

transferred maximally during the last two-thirds of gestation. Calcium, phosphorus, zinc, and iron are transported by active mechanisms. Copper passes from mother to fetus via a downhill gradient [4–6]. Placental transport of manganese and magnesium are poorly understood. The latter is probably translocated to the placenta utilizing a sodium/magnesium exchanger [7]. Placental size, surface area, blood flow, permeability, and metabolism affect transport of these nutrients [8].

Spontaneous twins occur in 1 of 80 pregnancies; of those, approximately 1/3 are monozygous and 2/3 are dizygous [9]. The development of a variety of assisted reproductive techniques has made twins and higher order multiple births more common. Discordance, defined as a difference in birthweight >10–15%, has been reported in almost one-third of all twin pregnancies [10]. Placental insufficiency is considered one of the more common causes of this discordance [11].

Accumulation of meconium begins in the distal intestine during the fourth month of gestation [12]. Essential elements are secreted into the gastrointestinal tract by the same processes as pancreatic and other gastrointestinal secretions. Since meconium stays in the gastrointestinal tract during the entire gestation, it may serve as an index of intrauterine mineral accumulation by the fetus. Widdowson et al. [13] and Kopito and Shwachman [14] were the first to study metal composition of meconium, but with limited discrimination of gestational age or birth weight. A recent investigation conducted in our institution showed that birth weight-adjusted concentrations of calcium, copper, iron, and phosphorus were higher in the meconium of 24–28-week gestational age infants than in the meconium of infants born at term [15]. Antonowicz and Shwachman [16] previously reported that the essential element composition of meconium from identical twins was similar, but that of fraternal twins was significantly different, although no quantitative data were provided.

We hypothesized that in multiple gestations, differences in birth weight would be reflected in the meconium accumulation of minerals. Specifically, in discordant twins, we presupposed that the heavier twin would have the greater concentration of essential elements in meconium and that the ratio of infant to placental weight would be related to the concentration of these minerals. Thus the lesser the infant/placental weight ratio, the greater the mineral concentration in meconium because a relatively larger placenta may be better equipped to meet the demands of the fetus. This study aimed to verify whether these hypotheses were supported by data obtained in a population of healthy newborn dizygotic twins.

MATERIALS AND METHODS

Meconium specimens were obtained from dizygotic twins born in North Shore University Hospital between January 1997 and February 1998. Dizygosity was ascertained from the pathologic report of the placenta. The population was entered in the study irrespective of sex or race and included term infants as well as prematures born at greater than or equal to 30 weeks of gestational age. Twenty-six pairs of twins were included in the study. The weight of the infants ranged from 1,300 g to 3,650 g and the percent discrepancy in weight, i.e., [(heavy twin weight − light twin weight)/heavy twin weight] × 100, ranged from 0.9% to 29.8%. The weight distribution of the twin pairs as a percentage of discrepancy and as a ratio between the heavy and the light twin is presented in Table I. Twins clustered in the 10.1–15.0% weight discrepancy,

TABLE I. Distribution of Body Weight Ratios in Twins and Their Weight Discrepancy

Percent discrepancy [heavy light twin weight] / heavy twin weight 100	No. of twins	Heavy/light weight ratio	No. of twins
0–5.0	5	1.00–1.10	10
5.1–10.0	5	1.11–1.20	7
10.1–15.0	6	1.21–1.30	2
15.1–20.0	1	1.31–1.40	5
20.1–25.0	4	1.41–1.50	2
25.1–30.0	4		
30.1–35.0	1		

corresponding to a weight ratio (heavy/light twin) of 1.11–1.20. The median discrepancy was 13.1%. This value was used to separate the twins into two groups: concordant (CC), with a weight discrepancy ≤13.1%, and discordant (DC), with a greater weight discrepancy. Thus there were 13 pairs of twins in each of the two groups. The mean (± SEM) weight of the CC group was 2.65 ± 0.09 kg and of the DC group was 2.17 ± 0.11 kg. Placental weights were obtained from the laboratory report, which decided the zygosity of the twins. Since the pathologic examination of the material did not include separation of the two hemiplacentas and approximate dimension values were not reliable indicators of size or weight, no attempts were undertaken to attach a partial placental weight to each of the twins in the pair. However, total placental weight was recorded.

Meconium was collected until appearance of transitional stools, i.e., when consistency and color changed to nonviscous and yellowish green. This usually took place within 3 days after birth. Meconium was scooped from diapers with a wooden spatula and immediately frozen at −20°C. The entire collection from each infant was combined, lyophilized from plastic containers, and weighed. The dried powder was triturated in a glass mortar and specimens (0.10–0.30 g) placed in test tubes and digested with 2.0 ml of concentrated nitric acid (trace element grade, Fisher Chem. Co., Pittsburgh, PA) at room temperature, until all material appeared to be dissolved. The acid solution was further diluted prior to analysis and filtered. Portions of the absorbent material of unused diapers were extracted with acid as described and used as a matrix blank to correct element concentration data. Samples were assayed for calcium, magnesium, iron, copper, zinc, and manganese by atomic absorption spectrophotometry (SpectrAA10, Varian Instruments, Sunnyvale, CA) against certified external standards (Fisher). Phosphorus was determined by a spectrophotometric method [17]. Results were expressed as μg/g of dry meconium and also as μg/g · kg birth weight. The latter correction was introduced to compensate for the possibility that mineral concentration increased with birth weight. Data were analyzed comparing the percent discrepancies and ratios between twin birth weights and the respective element concentrations in the meconium. In addition, we compared the values obtained in CC and the DC groups for all elements. The concentration of each element for the heavier and the lighter of each pair of twins was contrasted with the ratios of the respective birth weights by applying the nonparametric sign test [18]. Gender differences within and between groups were assessed by the Chi-square (χ^2) test. Relationships between infant birth weight and element concentration as well as weight differences between twins and element concentration differences in meconium were done by Pearson's r correlation test. We also compared the ratio in meconium element concentrations for heavy/light twins between the different elements. In addition, total placental weight was correlated with total birth weights of the twin pairs and also with the average element concentration in the meconium, corrected or uncorrected for birth weight. Infant/placental weight ratios were also contrasted against the average element concentration in the meconium of the twins. The threshold of significance was 0.05.

RESULTS

A comparison of element concentrations in the meconium of CC and DC twins, taken as a group, revealed that there were no differences for calcium, magnesium, phosphorus, iron, and zinc. However, the mean concentration for manganese in DC twins, corrected or uncorrected for birthweight, was higher than that of CC twins (Table II). Copper meconium concentration was also higher in DC than in CC twins when corrected for birth weight.

When the birth weight of both CC and DC twins was correlated with the metal concentration in meconium (Table III), there was a positive correlation for iron in the CC twins ($r = 0.475$, $P < 0.02$), and a negative correlation for magnesium ($r = -0.402$, $P < 0.05$) in the same group. A negative correlation for all twins was observed between infant weight of all twins and meconium copper ($r = -0.302$, $P < 0.05$).

The weight ratio between the heavier and the lighter twins, obviously always >1, and the corresponding concentrations of minerals in the meconium resulted in concordant >1 ratios for calcium, magnesium, iron, zinc, and manganese in the DC twins, i.e., the heavier twin presented with a higher concentration of the aforementioned elements in the meconium. In contrast, in the CC twins ratios >1 were not observed (Table III). Taking all twins together, the relationship between twin weight ratios and meconium element concentrations still applied for calcium, iron, zinc, and manganese.

The role of the placenta and its relationship with the newborn infant twins and their meconium metal content were examined from several standpoints. As expected, there was a very strong overall correlation between placental and infant weight ($r = 0.851$, $P < 0.0001$). When considering each group of twins separately, the coefficient of correlation was for CC and DC twins, $r = 0.875$ and 0.808, respectively, $P < 0.001$ (Fig. 1). Examination of total placental weight and average element concentration revealed that for DC twins, calcium concentration in the meconium increased as the total placental weight increased ($r = 0.650$, $P < 0.05$) (Table III). If metal concentration in the meconium was birth weight corrected, the relationship for calcium remained significant in the DC twins ($r = 0.601$, $P < 0.05$). In contrast, when all twins were considered, there was a negative correlation between total placental weight and birth weight-adjusted magnesium ($r = -0.509$, $P < 0.02$) and copper ($r = -0.565$, $P < 0.01$) concentrations. The other elements showed no correlation.

Infant to placental weight ratio appeared to bear significance with the concentration of calcium in meconium. Taking the total placental weight and the average calcium concentration in the meconium of both twins, there was a strong positive correlation between these two parameters ($r = 0.538$, $P < 0.01$). This relationship was obtained

TABLE II. Concentration of Metals in Meconium of CC and DC Twins (Uncorrected and Corrected for Birth Weight)

		Calcium	Magnesium	Phosphorus	Iron	Copper	Zinc	Manganese
Element conc. ± SEM (μg/g) N = 26	CC	3,588 ± 688	3,275 ± 319	264 ± 39	136 ± 25	87 ± 9	512 ± 54	28 ± 4
	DC	4,999 ± 1465	3,287 ± 278	233 ± 27	124 ± 9	108 ± 9	572 ± 158	45 ± 7*
Birthweight corrected element conc. ± SEM (μg/g · kg body weight) N = 26	CC	1,395 ± 272	1,317 ± 155	101 ± 16	49 ± 7	33 ± 4	204 ± 95	11 ± 2
	DC	2,217 ± 558	1,577 ± 155	113 ± 13	62 ± 6	52 ± 6**	297 ± 99	23 ± 5**

*$P < 0.05$; **$P < 0.02$ vs. CC twins.

TABLE III. Relationship Between Dizygotic Twin Somatic Characteristics and Placental Weight and Meconium Concentration of Essential Elements

		Calcium	Magnesium	Phosphorus	Iron	Copper	Zinc	Manganese
Correlation between weight of infants and mineral concentration	All	—	—	—	—	***	—	—
	CC	—	***	—	*	—	—	—
	DC	—	—	—	—	—	—	—
Correlation between heavy/light twin weight and meconium concentration[a]	All	**	—	—	**	—	*	*
	CC	—	—	—	—	—	—	—
	DC	*	*	—	*	—	*	*
Placental weight vs. element concentration	All	—	—	—	—	—	—	—
	CC	—	—	—	—	—	—	—
	DC	*	—	—	—	—	—	—
Placental weight vs. birthweight adjusted element concentration	All	—	****	—	—	****	—	—
	CC	—	—	—	—	—	—	—
	DC	*	—	—	—	—	—	—

[a]Significance of data by sign test.
Positive correlations: *$P < 0.05$, **$P < 0.01$; negative correlations: ***$P < 0.05$; ****$P < 0.01$.

even when the concentration of elements in meconium was corrected for birth weight ($r = 0.564$, $P < 0.01$). There was no correlation with other elements.

DISCUSSION

There are many factors that could cause growth discordance in twins. One factor is twin-to-twin transfusion, which is more prevalent in monozygotic than dizygotic twins [19]. By excluding monozygotic twins in our study population we minimized this factor. Another consideration that accounts for variation in birth weight is fetal gender, as males generally weigh more than females [20]. Since in our population 7 of the 13 pairs of CC twins and 6 of the 13 pairs of the DC twins had the same gender, this factor should have no bearing on our results. Also, there was no significant difference between the CC and the DC group in terms of gender since 16 of 26 infants in the CC group and 13 of 26 infants in the DC group were female ($\chi^2 = 0.312$, N.S.). Eliminating the above factors, we could, therefore, investigate placental causes of discordance and its role in nutrient transport.

Since meconium accumulates in the fetus from the sixteenth week of gestation [16], meconium may be an indirect indicator of the infant's intrauterine nutritional status in regard to stable components, such as mineral elements. From our data, we can infer that in discordant dizygotic (DC) twins, meconium metal concentration may be reflective of intrauterine nutrition since the heavier twin had a significantly higher meconium concentration of calcium, magnesium, iron, zinc, and manganese (Table III). This result confirms an earlier statement, unsupported by numerical data, that fraternal twins have significantly different meconium metal composition than identical twins [16]. This discrepancy may reflect an uneven distribution of essential

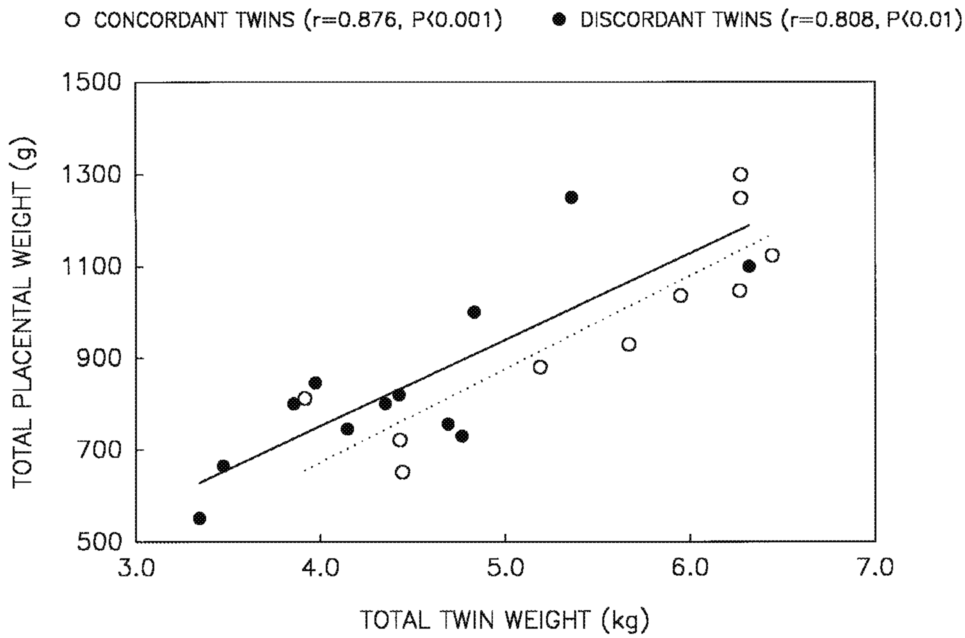

Fig. 1. Relationship between total twin weight and total placental for concordant (CC) twins ○, dotted line regression) and discordant (DC) twins ●, filled line regression). As indicated, both correlations were significant.

elements that have crossed the placental barrier between the twins. Additional factors that may play a role may be differences in fetal anabolic rate, assimilation of these nutrients, maternal dietary mineral intake, and pharmacologic administration of some salts, such as magnesium sulfate to the mother.

With the exception of manganese, the trace element analyzed in this study which is at the lowest concentration in meconium, there were no differences in other elements between CC and DC groups. When birth weight was applied as a correction, copper, as well as manganese, were higher in the meconium of DC twins (Table II). Since copper is transported across the placental by a downhill gradient [6], there may be a greater possibility of uneven distribution in the DC twins. The negative correlation found between meconium copper concentration and infant weight (Table III) may also be attributable to the absence of an active transport mechanism for this element.

For both the CC and DC twins, the greater the total placental weight, the heavier the combined infant weight (Fig. 1). There have been several possible physiologic explanations for this finding. Vetter [11] hypothesized a growth factor sequence stating that the smaller fetus has a less sufficient blood supply and thus produces a growth stimulating substance that results in increased growth of the other fetus with a normal placental function. Other factors such as the number of insulin receptors in the placenta have been found to correlate positively with the weight of the fetus [21]. Thus the placenta of the bigger twin would have a greater number of insulin receptors than that of the smaller twin, causing the former to grow more rapidly.

Aside from considering the total placental weight, we also examined the infant/

placental weight ratio. Although it has been found that infant/placental weight ratio is not an accurate marker of fetal growth [22], this index is considered to be a measure of the reserve capacity of the placenta, meaning the relative ability of the placenta to satisfy the demands of the fetus during gestation [23]. In the present study we did not find any significant correlation between the infant/placental weight ratio and meconium essential element content, suggesting that placental capacity was not a limiting factor in determining the ultimate appearance of minerals in meconium. It could be that since we were not able to obtain an accurate weight of each of the hemiplacentas and we used total placental weight, the infant/placental weight ratio results were less informative. Nevertheless, our findings correlate with a study in Nigeria suggesting that the occurrence of divergent birth weight is primarily associated with unequal sharing of placental mass [24].

In summary, the results obtained in this work, for dizygotic twins, are consistent with a greater access by the heavier infant to maternal essential mineral elements, or that this finding represents a greater volume of amniotic fluid material processing and excretory capacity. This is reflected in a generally higher meconium mineral concentration. This is clearly apparent in DC twins. Since there is a direct relationship between total placental and infant birth weight, placental sufficiency should determine the availability of mineral elements during gestation. However, the mechanisms underlying the ability of the placenta, as a function of its relative size, to deliver minerals to the fetus is a topic that remains to be fully explored.

Ultimately, meconium analysis may help in the assessment of mineral nutritional status of multiple birth infants and the early recognition of specific mineral deficiency states in these infants and may guide possible nutritional supplementation for the newborn.

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